

January 20, 1951.

Dear Mr. Loutit:

I CAN CERTAINLY SYMPATHIZE with your difficulties; every organism presents its own difficulties in this kind of work, particularly in the application of new and possibly unfamiliar techniques.

At this distance, it is difficult to think of all of the possible loopholes in your procedure, but I shall do my best. At the outset, however, it should be pointed out that some strains of bacteria have proven to be extremely refractory to attempts to isolate mutants. If it is essential to deal with *Pseudomonas pyocyaneus*, it may well be advisable to work with several different strains; if not, it has been a rather general experience that *E. coli* is probably the most convenient organism for biochemical genetic work.

The most obvious feature of your technique that should be scrutinized is the lack of an interfering cultivation of your bacteria, in a complete medium, between the irradiation and the screening on agar. This probably has two functions: a) to allow the separation of mutant and non-mutant nuclei within a single cell into separate daughter cells, and b) especially in your material, from your description, to equalize irregular physiological disturbances which result in variable lags of further growth in the treated bacteria. Very likely, the mutants which may be present after irradiation may be obscured, for the purposes of your delayed enrichment detection procedure, by this variability. I would therefore recommend that your treated populations be allowed to grow in a complete medium for three or four generations (i.e., ca. 10-fold increase) before they are plated out. This does make more difficult any quantitative analysis of yields of mutants as functions of X-ray dosages, and so forth, but there is no help for it, and for this reason, the production of auxotroph mutants is not a very satisfactory approach for quantitative work. But if you are interested in the mutants themselves, this does not much matter.

It is also possible that you are "over-killing" the bacteria. I would recommend that you adjust either the initial count or the X-ray dose so that you will have a larger sample of survivors, especially if you introduce an intermediate cultivation. If the mutants are your main interest, I also think that you will find ultra-violet light to be generally a more amenable mutagen.

I have had some luck in producing mutants of *P. fluorescens* with the "penicillin" method, although it was, of course, necessary to use very high unitages (1000 u/ml) in order to lyse the bacteria.

Possibly you may have overlooked some papers detailing the methodology of biochemical mutation study in bacteria. May I refer you to Davis' review in *Experientia*, 6:41-50 Feb. '50, and to a joint effort in *Methods in Medical Research*, Vol. III, 1950 (Year Book Publishers, Chicago)?

I shall be interested to hear whether these suggestions have been of any empirical use.

Yours sincerely,

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Associate Professor of Genetics